

Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing  
Chicken Embryo-Report of the in-house investigation of **Monosodium Glutamate** in the  
developing chicken embryo 4/20/76

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# MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION

TO GRAS Review Branch, HFF-335

DATE: April 20, 1976

Dr. Herbert Blumenthal, Acting Director  
Division of Toxicology, HFF-150

FROM M. Jacqueline Verrett, Ph.D.   
Reproductive Physiology Branch, HFF-157

SUBJECT Investigation of the Toxic and Teratogenic Effects of GRAS Substance to  
the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Monosodium  
Glutamate in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of  
GRAS Substances to the Developing Chicken  
Embryo: Monosodium Glutamate

Protocol:

Monosodium Glutamate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

#### Discussion:

Monosodium Glutamate displayed no toxicity up to 200 mg/kg when administered via the air cell and no LD<sub>50</sub> could be calculated. Yolk treatment resulted in slight toxicity only, and when administered at 96 hours an LD<sub>50</sub> estimate of 357.58 mg/kg (17.88 mg/egg) was obtained.

Scattered abnormalities were observed for all four test conditions but in no instance were the serious abnormalities higher in incidence than or different from those observed in the background. Monosodium Glutamate displayed no teratogenicity under the test conditions employed.

1. Monosodium Glutamate, Accent International Inc., San Jose, Calif., Lot No. 72-325
2. McLaughlin, J., Jr., Marliac, J. P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770
3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002-1006
4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Monosodium Glutamate  
Air Cell at 0 Hours

Table 1

Dose		Number of Eggs	Percent** Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.00	200.00	144	31.94	3.47	0.69
5.00	100.00	144	27.08	3.47	0.00
2.50	50.00	145	31.72	6.20	1.37
1.250	25.00	144	27.77	4.16	2.08
0.500	10.00	175	26.28	1.14	0.57
Water	--	134	27.61	2.23	0.74
Control	--	415	14.93	1.44	1.20

\*\*Slope not significantly different from zero  $p=0.05$

Monosodium Glutamate  
Air Cell at 96 Hours

Table 2

Dose mg/egg    mg/kg		Number of Eggs	Percent ** Mortality	Percent Abnormal	
				Total	Structural
5.00	100.00	124	21.77	3.22	2.41
2.50	50.00	125	16.80	2.40	2.40
1.250	25.00	124	23.38	4.83*	2.41
0.6250	12.50	124	20.96	2.41	1.61
0.2500	5.00	125	20.80	1.60	0.00
Water	--	125	14.40	0.00	0.00
Controls	--	415	14.93	1.44	1.20

\*\*Slope is negative

\*Significantly different from solvent  $p \leq 0.05$

Monosodium Glutamate  
Yolk at 0 Hours

Table 3

Dose		Number of Eggs	Percent Mortality	Percent	
mg/egg	mg/kg			Total	Abnormal Structural
10.00	200.00	144	69.44*	2.08	0.00
5.00	100.00	145	67.58*	0.68	0.68
2.50	50.00	144	70.13*	0.00	0.00
1.250	25.00	144	65.97*	1.38	0.00
0.500	10.00	115	50.43*	0.00	0.00
Water	--	145	31.72	3.44	1.37
Controls	--	415	14.93	1.44	1.20

\*\*Slope not significantly different from zero  $p=0.05$

\*Significantly different from solvent  $p \leq 0.05$

Monosodium Glutamate  
Yolk at 96 Hours

Table 4

Dose		Number of Eggs	Percent** Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.00	100.00	105	53.33*	0.00	0.00
2.50	50.00	103	56.31*	2.91	0.00
1.250	25.00	105	52.38*	7.61*	3.80
0.6250	12.50	104	52.88*	3.84	0.96
0.2500	5.00	105	41.90*	2.85	0.95
Water	--	110	22.72	0.90	0.00
Controls	--	415	14.93	1.44	1.20

\*\*LD<sub>50</sub> 357.58 mg/kg (17.88 mg/egg)

\*Significantly different from solvent  $p \leq 0.05$